United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract

Government Performance Results Act (GPRA) Goal 8
Annual Performance Measure 68

Significant Research Findings:

Report on Evaluation of Molecular Biological Indicators of Exposure in Fish to Pesticides Based on Experiments in Artificial Ecosystems

Scientific Problem and Policy Issues

The vulnerability of aquatic wildlife to pesticides at the watershed and regional scales remains to be determined. An important component of a vulnerability assessment, the distribution of pesticides across a number of regions, was made clearer in March 2002 when the U.S. Geological Survey (USGS) published a report on the occurrence of a number of chemicals in surface waters across the U.S. at environmentally relevant concentrations. Many pesticides were measured in this study including the modern, moderately persistent herbicides alachlor and atrazine. Presently, risk assessments of these pesticides are based on data from laboratory toxicity studies and from exposure models that predict fate and transport into streams and lakes under certain application conditions and from bioaccumulation models. Actual risk of pesticides to aquatic wildlife is still difficult to determine since studies have not reported on another component of vulnerability, the pesticides' bioavailability. Bioavailability refers not only to the measurement of chemicals or their metabolites in the plasma of fish, but, ideally, their interaction with the cellular biomolecules to begin to bring about significant biological changes within the organism.

Research Approach

The primary objective of this study was to study the early biological responses of fish following exposure to pesticides in mesocosms, or surrogate ecosystems, and to develop and evaluate indicators of bioavailable pesticides for use in regional field studies. A number of molecular indicators of exposure were chosen to study bioavailability based on reports of the estrogenic, mixed function oxidase-inducing and genotoxic activities of the chloroacetanilide and triazine herbicides. Respectively, these indicators were liver vitellogenin gene expression in male common carp (*Cyprinus carpio*), liver cytochrome P450IA1 gene expression and DNA single strand breaks in bloods cells as measured with single cell gel electrophoresis methods. The herbicides studied in separate experiments were alachlor and atrazine.

Results and Impact

Chemical analyses of alachlor, atrazine, nitrogen and phosphorus in the mesocosms confirmed the experimental conditions as being oligotrophic and

confirmed that the attenuation rate of alachlor and atrazine matched previous results from studies at the University of Kansas, establishing that conditions that were reproducible and allow for the control of parent compound and metabolite concentrations in future mixture experiments.

In spite of reports of the weak estrogenic activity of both alachlor and atrazine, no changes in vitellogenin gene expression were observed in male carp exposed to these herbicides. However, increases in expression of liver cytochrome P450IA1 were observed. Interestingly, both herbicides, characterized as weak and variable mutagens in controlled studies with laboratory rodents, were shown to produce dose-responsive increases in DNA strand breaks at low and environmentally relevant doses (20-50 ppb range). In general, these data contribute to the considerations for reregistration of atrazine whose genotoxicity has been questioned.

Overall, these studies represent some of the first tests anywhere exploring changes in molecular diagnostic indicators following exposure to modern pesticides. Biological activities associated with some endocrine disruptors were measured (estrogenicity, mixed-function oxidase-inducibility and genotoxicity) and we can now report clear changes or lack of change in all three instances. Perhaps more significantly, these studies showed the feasibility of conducting ecologically realistic experiments with aquatic stressors at environmentally relevant concentrations in a controlled manner. This, and other studies, both within this organization and in academia, comprise a body of work establishing molecular indicators in highly relevant exposure studies, breaking new ground in ecological risk assessment.

These diagnostic indicator development studies address the Goal 8.1 Multiyear Plan Programmatic Long-Term Goal (LTG) for Ecological Protection:

> LTG 2: “Diagnosis and Forecasting Research”:“Federal, State and Local managers can diagnose cause and forecast future condition in a scientifically defensible fashion to more effectively protect and restore valued ecosystems.”
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> 2006 Annual Performance Goal (APG):“Risk assessors can use improved/new diagnostic indicators to determine causes of ecological impairment.”
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> 2002 (Annual Performance Measure (APM):“New molecular diagnostic indicators produced.”

Research Collaboration and Research Products

These studies were conducted as part of a cooperative agreement with Dr. David Graham at the University of Kansas Department of Civil and Environmental Engineering. Development of the oligotrophic mesocosms and herbicide dosing and chemical analyses were performed by Dr. Graham’s colleagues. Scientists from the National Exposure Research Laboratory’s Ecological Exposure Research

Division at the U.S. Environmental Protection Agency's Office of Research and Development deployed caged fish in the mesocosms, sampled fish after exposure and completed the molecular analyses. The research has been presented at a scientific conference:

Chang, L.W., Meier, J.R., Graham, D.W. and Toth, G.P. Evaluation of Genetic Damage in Fish Exposed to Pesticides in Field Aquatic (Presented at the 2000 Environmental Mutagenesis Society Meeting).

A manuscript is being submitted for publication in *Environmental Toxicology and Chemistry*.

Future Research

The conduct of these mesocosm studies opened the door for consideration of a number of future aquatic stressor bioavailability studies. Perhaps most significantly, caged organisms can be repeatedly deployed in the mesocosms to bioavailability and attenuation of pesticides in exposure studies. Data from this type of experiment would enhance the relevance of pesticide fate and transport studies. The additional study of mixtures in mesocosms with repeatedly deployed organisms will allow for the testing of assumptions currently made about aggregate exposure and interactions between pesticides.

Contacts for Additional Information

Questions and inquiries on NERL's molecular indicator development and mesocosm research can be directed to:

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